REMARKS

Claims 1-75 are currently pending in the application. Claims 74 and 75 are withdrawn from examination. Claims 1-17, 20, 36, 50-61 and 64 are cancelled herein without prejudice. Claims 18, 21, 22, 26, 34, 37, 42, 62, 65 and 69 are proposed to be amended herein. Upon entry of the amendments proposed herein, claims 18, 19, 21-49, 62-63 and 65-73 will be under examination.

Claim 18 is proposed to be amended to recite, before the detecting step (which becomes step c), step (b) of "separating distinctly sized amplification products by size and/or by charge." Support for the amendment is found, for example, in previously examined dependent claim 20 (cancelled herein).

Claims 21, 37 and 65 are proposed to be amended simply to change the dependency from a cancelled claim to a pending claim. Claims 26, 42 and 69 are amended so that specific parts of independent parent claims referred to are consistent with the amendments to those parent claims.

Claim 22 is proposed to be amended to change the term "comprising" to "comprises" merely for grammatical correctness.

Claim 34 is proposed to be amended to add the term "second" to the term "tag sequence" in line 14 of the claim in order to agree with the antecedent reference to "second tag sequences" at line 6. Claim 34 is also proposed to be amended to add the step, after step (a), of "separating distinctly sized amplification products by size and/or by charge." The amendment incorporates the limitation originally recited in examined dependent claim 36 (cancelled herein).

Claim 62 is amended to recite new step VIII), of "separating distinctly sized amplification products by size and/or by charge" and amends the language of step IX) (formerly step VIII) to recite "detecting incorporation of at least one distinguishable label in distinctly sized amplification products, thereby determining the identities of the nucleotides present at said known polymorphic sites." The amendments add the limitation regarding separation of distinctly sized amplification products as recited, e.g., in examined claim 64 (cancelled herein).

Claim 65 is amended to correct dependency from cancelled claim 64 to independent claim 62.

The amendments introduce no new matter. Further, the limitations introduced to the independent claims by the proposed amendments have been considered within the context of

dependent claims. As such, the amendments do not raise issues requiring a new search. Entry and consideration of the amendments is respectfully requested.

Rejections under 35 U.S.C. §102:

Claims 1, 2, 5, 10, 12, 18-19, 22, 27, 34-35, 38 and 43 are rejected under 35 U.S.C. 102(b) as being anticipated by Myakishev et al. (Genome Research (Jan. 2001) 11: 163-169; cited previously). With regard to independent claim 1, the Office Action states:

> Regarding claim 1, Myakishev teaches a method of determining a given nucleic acid sample, the identity of the nucleotide at a known polymorphic site (see Figure 2 and Results section, pages 163-165), said method comprising:
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> a) subjecting to an amplification regimen a population of primer extension products generated from a nucleic acid sample (Figure 2, top panel, where extension with the tailed allele-specific primers occurs in round 1 of the amplification to generate the population of primer extension products; see also page 164, col. 2; steps 3-4 shown in Figure 2 and page 164, col. 2 teach amplification of these primer extension products),
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> each primer extension product comprising a tag sequence, which tag sequence specifically corresponds to the presence of one specific nucleotide at a known polymorphic site (page 164, column 1, teaches that the allele-specific primer contains a different 21-base tail at the 5'end),
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> wherein said amplification regimen is performed using an upstream amplification primer (the "reverse primer" of page 163, col. 2 and page 164, col. 2) and a set of distinguishably labeled downstream amplification primers (the two energy-transfer (ET) primers of Fig. 2, see also page 164, col. 2), each member of said set of downstream amplification primers comprising a said tag sequence comprised by a member of said population of primer extension products and a distinguishable label (see Fig. 1 for the structure of the ET primers), wherein each distinguishable label specifically corresponds to the presence of a specific nucleotide at said polymorphic site (Fig. 2 teaches red and green labels for different mutations)
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> b) detecting incorporation of a distinguishable label into a nucleic acid molecule, thereby to determine the identity of the nucleotide at said polymorphic site (see Fig. 2 and pages 164-165).

With regard to independent claim 18, the Office Action states:

Regarding claim 18, Myakishev teaches a method of determining, for a given nucleic acid sample, the identities of the nucleotides at a set of known polymorphic sites to be interrogated (see Fig. 2 and pages 163-165; note that 9 SNPs were tested), said method comprising:

a) subjecting to an amplification regimen, a population of primer extension products generated from a nucleic acid sample (Figure 2, top panel, where extension with the tailed primers occurs in round 1 of the amplification to generate the population of primer extension products; also page 164, col. 2; steps 3-4 shown in Figure 2 and page 164, col. 2 teach amplification of these primer extension products),

each primer extension product comprising a member of a set of tag sequences, which tag sequence specifically corresponds to the presence of one specific nucleotide at a known polymorphic site (page 164, col. 1 teaches that the allele-specific primers contain different 5'tags),

wherein said amplification regimen is performed using one upstream amplification primer for each sequence comprising a known polymorphic site to be interrogated (the reverse primer, page 163), and a set of distinguishably labeled downstream amplification primers (the ET primers; Fig. 2),

each member of said downstream amplification primers comprising a said tag sequence comprised by a member of said population of primer extension products (see Figure 1) and a distinguishable label that specifically corresponds to the presence of a specific nucleotide at said polymorphic site (see Figures 1 & 2), and where said upstream amplification primers are selected such that each polymorphic site of said set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product (see page 164, col. 1, where the upstream primer was hybridized at different points on the template to produce differently sized amplicons)

b) detecting incorporation of a distinguishable label in distinctly sized amplification products, thereby to determine the identity of the nucleotide at each said polymorphic site (see Fig. 2 and also the Methods section, page 168, where fluorescence is detected).

With regard to independent claim 34, the Office Action states:

Regarding claim 34, Myakishev teaches a method of determining, for a given nucleic acid sample, the identities of the nucleotides at a set of known polymorphic sites to be interrogated (see Fig. 2 and pages 163-165; note that 9 SNPs were tested), said method comprising:

a) subjecting to an amplification regimen, a population of primer extension products generated from a nucleic acid sample (Figure 2 and page 164, col. 2), each primer extension product comprising a first tag sequence (generated by extension of the reverse primer) or its complement and a member of a set of second tag sequences or its complement (the 21 nt tail added in step 1 of the reaction), the presence of which second tag sequence or its complement specifically corresponds to the presence of one specific nucleotide at a known polymorphic site (page 164, col. 1), wherein for each polymorphic site in said set of polymorphic sites, said first tag sequence is located at a distinct distance of 5' of said polymorphic site, relative to the distance of said first tag sequence from a polymorphic site on molecules in said sample containing other polymorphic sites (page 164, col. 1 teaches that the reverse primer may hybridize at different positions on the template), wherein said amplification regimen is performed using an upstream amplification primer comprising said first tag sequence (the reverse primer, page 163), and a set of distinguishably labeled downstream amplification primers (the ET primers), each member of said set of downstream amplification primers comprising a said tag sequence comprised by a member of said population of primer extension products (the tail sequence; see Fig. 1) and a distinguishable label that specifically corresponds to the presence of a specific nucleotide at said polymorphic site (see Fig. 1 and 2), and wherein said upstream amplification primers are selected such that each polymorphic site of said set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product (page 164, col. 1 teaches hybridization of the reverse primer at different locations on the template)

b) detecting incorporation of a distinguishable label in distinctly sized amplification products, thereby to determine the identity of the nucleotide at each said polymorphic site (page 168, col. 1).

From this, the Office Action concludes that independent claims 1, 18 and 34 and claims 2, 5, 10, 12, 19, 22, 27, 35, 38 and 43 which respectively depend from them are anticipated by Myakishev et al. Applicant respectfully disagrees.

First, while Applicant maintains that the invention of claim 1 and claims dependent from it are novel over Myakishev et al., the rejection of these claims is rendered moot by their cancellation herein without prejudice.

With regard to claims 18 and 34, Applicant submits first that the Myakishev et al. reference does not teach a method in which "the identities of the nucleotides at a set of known polymorphic sites" are interrogated in one reaction as set out in claims 18 and 34. While Myakishev et al. states that "it is both more economical and more accurate to perform both allele specific amplifications in

a single tube, rather than in separate tubes," (see p. 165, col. 2) the reference is describing the detection of both alleles of a *single* polymorphism in the same tube. The reference does not teach how to detect alleles of more than one SNP target in a single reaction. Specifically, the Myakishev et al. reference refers to Figure 5, stating

> "Figure 5 shows an experiment in which DNA from an A/A homozygote and from a T/T homozygote for an A/T SNP were assayed either by testing each DNA in two separate tubes – one containing the allele-specific and ET primer for A and the other containing the allele-specific and ET primer for T (left panel) – or in a single tube with both sets of primers (right panel)."

In each instance, however, the same SNP was interrogated – i.e., the SNP is "an A/T SNP," and the assay detected, at most, both the A and T alleles of that SNP in one reaction. More than one polymorphism, i.e., a *set* of known polymorphic sites, is not interrogated in one reaction as required in the method of claims 18 and 34. As discussed in the accompanying Rule 132 Declaration of Dr. Vladimir Slepnev, the Myakishev et al. method would not readily adapt to the detection of a *set* of known polymorphisms in a single reaction because such a multiplex assay would require multiple distinguishable fluorescent labels per polymorphism – one for each allele (i.e., there must be at least two distinguishable labels for each polymorphic site, and more would be required if a third or fourth nucleotide possibility existed for a given site). As discussed in the Declaration, in order to be distinguishable, the excitation and emission spectra for each member of each such pair would have to be sufficiently distinct from all other labels so as to avoid interference, quenching or artifactual signals caused by the interaction of the fluorescent labels. Myakishev et al. teaches only two labels, fluorescein (green) and sulforhodamine (red). The reference does not teach an assay to interrogate "the identities of the nucleotides at a set of known polymorphic sites" in one reaction as required in claims 18 and 34.

Further with regard to claims 18 and 34 as proposed to be amended, the Myakishev et al. reference does not teach separation of distinctly sized amplification products. Myakishev et al. describes a homogeneous assay in which results are read by a fluorescence plate reader without any separation of distinctly sized amplification products.

Also with regard to claims 18 and 34, the Myakishev et al. reference does not teach a method "wherein said upstream amplification primers are selected such that each polymorphic site

of said set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product." The Office Action points to page 164, column 1 "where the upstream primer was hybridized at different points on the template to produce differently sized amplicons" as teaching this limitation. This is incorrect for at least two reasons.

First, at the passage cited by the Office Action, Myakishev et al. teaches that:

"The distance between the allele-specific and reverse primers *was not critical* and varied from 7 to 157bp in our experiments." (Emphasis added)

That is, Myakishev et al. specifically teaches that the distance between the primers, and therefore, the length of the amplicons, "is not critical." This is directly counter to the claimed method, which requires that "upstream amplification primers are selected such that each polymorphic site of said set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product." Because, as the reference states, the size is not critical in Myakishev et al., the method taught by the reference does not require primers "selected such that each polymorphic site of said set of known polymorphic sites to be interrogated corresponds to a distinctly sized amplification product" as required by both claims 18 and 34. That is, if size "is not critical," the method cannot teach any requirement for distinctly sized amplification products.

Second, where Myakishev et al. states that the distance "was not critical," and notes that the distance "varied from 7 to 157 in our experiments," the reference never includes primers that amplify distinctly sized amplicons in the *same* amplification regimen as required by each of claims 18 and 34. Applicant notes that even where both alleles of a single SNP were assayed in one reaction in the Myakishev et al. reference, there is no indication that the different amplified products differed in length. Applicant further notes that the length of the amplicon in Myakishev et al. "is not critical" at least in part because only fluorescence is read in a plate reader according to the reference – the assay is a homogeneous assay based on fluorescence energy transfer, and does not involve *any* detection of size, or detection of a set of distinctly sized amplification products produced in one amplification.

Also with regard to claims 18 and 34, the Office Action states that Myakishev et al. teaches a method for determining, for a given nucleic acid sample, the identities of the nucleotides at a set of known polymorphic sites to be interrogated, citing Figure 2 and pages 163-165, stating "note that

9 SNPs were tested." While the reference may have examined nine different SNPs over the course of the reported experiments, they were not examined in the same reaction. That is, at no point in the Myakishev et al. reference were more than *one* SNP genotyped using one amplification regimen. This is in contrast to claims 18 and 34 which require determining the identities of the nucleotides at a set of known polymorphisms in an amplification regimen. Separately testing nine separate SNPs in separate reactions is not the same as testing a set of known polymorphisms in an amplification regimen as required by the claims.

Considering any one or all of the distinctions of both claims 18 and 34 over the teachings of the Myakishev et al. reference, Applicant submits that the Myakishev et al. reference does not teach the invention claimed in either of claims 18 and 34, or in claims that depend from them.

In view of the above, Applicant submits that Myakishev et al. fails to teach all elements recited in either of independent claims 18 or 34. Thus, the reference cannot anticipate any of these claims or, by extension, claims that depend from them. Reconsideration and withdrawal of the §102 rejection of these claims over Myakishev et al. is respectfully requested.

Rejections under 35 U.S.C. §103:

Myakishev et al. in view of Piggee et al.

Claims 3, 4, 9, 20, 21, 26, 36, 37 and 42 are rejected under 35 U.S.C. §103(a) as being obvious over Myakishev et al. in view of Piggee et al. The Office Action states:

> Myakishev teaches the method of claims 1, 18 and 34, as discussed above.
> Myakishev teaches fluorescence detection of amplified products rather than capillary electrophoresis separation and detection.
> Regarding claims 9, 26 and 42, Myakishev teaches a thermal cycling device with a fluorescence detection system (Methods, page 168). Note that the specification does not require that the elements of the modular apparatus of claim 9, 26, and 42 (a thermal cycling device, a sampling device, a capillary electrophoresis device and a fluorescence detector) be physically connected.
> Regarding claims 3-4, 20-21, 36, and 37, Piggee teaches a method of detecting point mutations using capillary electrophoresis and laser-induced fluorescence detection (see abstract).
> Regarding claims 9, 26, and 42, Piggee teaches a capillary electrophoresis device with a sampling device, and a fluorescence detection system (pages 368-369)."

Applicant respectfully disagrees.

The rejection is rendered moot with regard to claims 3, 4 and 9 by the cancellation of those claims without prejudice herein.

Applicant notes that while the methods of claims 18 and 34 each require amplification, the detection method of Piggee et al. does not amplify anything. This is because Piggee et al. specifically uses single base incorporation of labeled dideoxynucleotide terminators (see the Abstract). Once a terminator is added onto a primer in the Piggee et al. method, that primer can no longer be a substrate for further additions, with or without cycling – thus, there is no amplification in the Piggee et al. detection method. The "touchdown" cycling taught by Piggee et al. is used, according to the reference, "to rapidly cover a range of annealing temperatures without requiring the optimization of each annealing temperature" – that is, cycling is not used to and does not amplify a product in the Piggee et al. assay, in contrast to the requirements of each of the claimed methods.

Applicant understands that the Office Action cites Piggee et al. for the use of capillary electrophoresis, and not necessarily for amplification. However, the Office Action also cites Piggee et al. as teaching facilitation of multiplexing "through the use of different length primers." As discussed above, the detection method of Piggee et al. does not amplify anything, and as such, even where Piggee et al. may teach an approach for multiplexing, Piggee et al. does not teach multiplex amplification or a method which would perform interrogation of multiple SNP sites within the same amplification regimen.

Importantly, the use of different length primers as taught by Piggee et al. will not necessarily result in distinctly sized amplification products as required by the claims. This, taken together with Myakishev et al.'s teaching that "the distance between the allele-specific and reverse primers was *not critical*" means that neither Myakishev et al. nor Piggee et al. teaches the "distinctly sized amplification products" produced in an amplification regimen as required by claims 18 and 34. As discussed above with regard to the §102 rejection, Myakishev et al. *never* performs multiplex amplification. As also discussed above, the multiplex detection mentioned by Piggee et al. does not involve amplification at all, and certainly not amplification that results in "distinctly sized

amplification products" produced in an amplification regimen as required by the subject claims. Thus, even where Piggee et al. may refer to multiplex detection, the method taught by Piggee et al. for such multiplex detection is not that required in the claimed methods and the combination does not render the invention of claims 18 and 34 as amended or claims that depend from them obvious.

Also with respect to the Piggee et al. reference, Applicant notes that claims 28, 44 and 73, which depend from independent claims 18, 34 and 62, respectively, recite the use of the same set of downstream amplification primers to amplify members of a set of different, known polymorphic sequences. In each instance, the set of distinguishably labeled downstream amplification primers consists of: a subset that comprises a tag sequence that specifically corresponds to the presence of A at the polymorphic site; a subset that comprises a tag sequence that specifically corresponds to the presence of C at the polymorphic site; a subset that comprises a tag sequence that specifically corresponds to the presence of G at the polymorphic site; and a subset that comprises a tag sequence that specifically corresponds to the presence of T at the polymorphic site. In this embodiment, a single downstream amplification primer will amplify those different members of the set of known polymorphic sequences that, for example, have an A at the polymorphic site. Another downstream amplification primer will amplify those different members of the set of known polymorphic sequences that, for example, have a C at the polymorphic site, and so on. Thus, in this embodiment, a *primer of the same length* (e.g., the primer with a tag sequence that specifically corresponds to the presence of A at the polymorphic site) will amplify multiple different polymorphic sequences, in contrast to the teaching of Piggee et al. that primers of *differing* lengths would be required to detect multiple different polymorphisms. As discussed, Myakishev et al. does not teach multiplexing. Thus, the methods of dependent claims 28, 44 and 73 are further distinguished over those taught by Piggee et al., alone or in combination with Myakishev et al.

Reconsideration and withdrawal of the §103 rejection over the combination of Myakishev et al. and Piggee et al. is respectfully requested.

Myakishev et al. in view of Wiesner et al. and Piggee et al.

Claims 6-8, 23-25 and 39-41 are rejected under 35 U.S.C. §103(a) as being obvious over Myakishev et al. in view of Wiesner et al. and Piggee et al. The Office Action states:

> Myakishev teaches the method of claims 1-2, 5, 10, 12, 18-19, 22, 27, 34-35, 38 and 43 as discussed above.
>
> Myakishev does not teach analyzing an aliquot of the PCR during the reaction process. Also, Myakishev does not teach capillary electrophoresis.
>
> Wiesner teaches a method of determining the initial concentration of a PCR template by measuring the amount of product accumulating in consecutive cycles (see abstract).
>
> Regarding claims 6-7, 23-24, 39 and 40, Wiesner teaches taking an aliquot after each cycle of a PCR reaction and analyzing the amount of accumulating product by agarose gel electrophoresis and scintillation counting (page 554, Methods section). Wiesner teaches that by analyzing aliquots the need for standardization of the signal due to differing initial product concentrations is eliminated. The teachings of Wiesner also permit optimization of cycling parameters and an early identification of the reaction product (page 555, where the product is detectable halfway through the amplification reaction.
>
> Wiesner does not teach analysis using capillary electrophoresis.

From this, the Office Action concludes:

> It would have been prima facie obvious for one of ordinary skill in the art at the time of the invention to incorporate the teachings of Wiesner and Piggee into the genotyping method of Myakishev. Wiesner particularly pointed out the advantages of taking an aliquot after each cycle of the amplification reaction, namely: the ability to rapidly optimize the reaction conditions, to quickly determine the identity and quantity of the product, and also determine the initial concentration of the template for standardization purposes (see above). The ordinary practitioner would have been motivated by the teachings of Wiesner to analyze an aliquot of the PCR reaction of Myakishev in order to incorporate the above advantages into the method. Furthermore, the ordinary practitioner would have been motivated by the teachings of Piggee to substitute capillary electrophoresis for the agarose electrophoresis and scintillation counting taught by Wiesner. As discussed above, Piggee taught that capillary electrophoresis offered a rapid, radiation-free, automation-friendly, and high-throughput method for analyzing DNA samples (see above). The ordinary artisan, interested in combining the teachings of Myakishev and Wiesner in order to more rapidly detect mutations, would have been further motivated to use capillary electrophoresis, as suggested by Piggee, in order to further increase the speed, multiplexing ability, automation and throughput of the assay. Therefore, the combined teachings of Myakishev, Wiesner, and Piggee result in the instant claims 6-8, 23-25, and 39-41.

Applicant respectfully disagrees.

The rejection is moot with respect to claims 6-8 due to the cancellation of those claims without prejudice herein.

With respect to parent claims 18 and 34 and claims dependent from them, Applicant notes that the Wiesner et al. reference does not remedy any of the defects of the Myakishev et al. reference discussed herein above with regard to the novelty rejections of claims 18 and 34, nor does it remedy the defects of a combination of Myakishev et al. and Piggee et al. Specifically, Wiesner et al. does not teach a method of determining, for a given nucleic acid sample, the identities of the nucleotides at *a set of known polymorphic sites* to be interrogated, in an amplification regimen. As discussed above, Piggee et al. also fails to remedy the defects of Myakishev et al. regarding claims 18 and 34 with respect to determining the identities of the nucleotides at *a set of known polymorphic sites* to be interrogated in an amplification regimen. Thus, no combination of Myakishev et al., Piggee et al. and Wiesner et al. can render obvious the invention of claims 18 or 34. If the references cited fail to teach all elements of the parent independent claims, they cannot teach all elements of the dependent claims. Thus, none of claims 18, 23-25, 34 or 39-41 can be obvious over the cited combination of references.

With respect to dependent claims 25 and 41, Applicant further submits that the Wiesner et al. method cannot be adapted for use with capillary electrophoresis as required by those claims. The controlling premise of Wiesner et al. is that the method described by the reference permits the quantitation of the initial concentration of template. However, as discussed in detail in Dr. Slepnev's Declaration, in order to reap the quantitative benefits that are central to the teachings of Wiesner et al., one must know how much, i.e., what *volume*, of an amplification reaction is subjected to separation and detection. As explained in Dr. Slepnev's Declaration, the volume of sample applied to a capillary for capillary electrophoresis is not known, particularly if one uses, for example, electrokinetic injection, as taught by the Piggee et al. reference.

Quantitation of the initial amount of template in a sample is the central axis of Wiesner et al. See, for example, the abstract (termed the "Summary") for the Wiesner et al. reference, which

states, as its first sentence, "In this report, we show that *the actual number* of target molecules of the polymerase chain reaction can be determined by measuring the concentration of product accumulating in consecutive cycles" (emphasis added). Further, in the first two paragraphs of the reference, Wiesner et al. states:

> "Protocols have been reported comparing the accumulation of a PCR-product derived from the target sequence to a product derived from a standard, whose concentration is known (2, 3). Because of tube effects, both standard and unknown sample ideally should be amplified within the same reaction, and in order to obtain similar amplification efficiencies, they should resemble each other as close as possible. Consequently, small differences in size (4) or in sequence, generating a new restriction site (5, 6), were introduced into the standard to allow separation of the two products.. *Thus, synthesis of standard is laborious, and more important, an exact calibration of the standard solution is imperative, but prone to systematic errors, which will lead to wrong results.*
>
> While studying the biogenesis of mitochondria, especially the question whether mitochondrial (mt) gene expression is regulated at the level of transcription or by changing the copy number of mt DNA, as postulated by others (7, 8), we have developed a PCR-based method to *determine* ***the actual number*** *of mt DNA molecules in crude tissue extracts without using a standard.* This highly sensitive method can be used for quantitation of any DNA or RNA template in a wide variety of questions." (emphases added)

Given this emphasis in the Wiesner et al. reference on determining "the actual number" of target DNA molecules in a sample, without a standard, Applicant submits that the quantitative aspect of the Wiesner et al. reference is the operating principle of the reference. However, as discussed in Dr. Slepnev's Declaration, the Wiesner et al. method necessarily depends upon knowledge of the amount of sample separated on the agarose electrophoresis gel. As explained in Dr. Slepnev's Declaration, such knowledge of sample volume is not generally possible in capillary electrophoresis. Thus, as discussed by Dr. Slepnev in the Declaration, *Wiesner et al.'s quantitation method will not work when capillary electrophoresis is used for separation of amplified product.* One simply cannot solve Equation 1 of the Wiesner et al. reference without knowing the volume of sample loaded on the gel. Because the quantitative aspect of Wiesner et al. is central to its teachings, no combination in which its quantitative aspect is necessarily changed can render the claimed invention obvious. See, e.g., *In re Gordon*, 733 F.2d 900 (Fed. Cir. 1984) (also cited in the

MPEP 2143.02(V)), in which claims to a blood filter device were rejected by the Board of Patent Appeals and Interferences as obvious over a gasoline filter device taught by the French reference, which had to be turned upside down in order to satisfy the limitations of the claims. In overturning the obviousness decision of the Board, the Court of Appeals for the Federal Circuit stated "Indeed, if the French apparatus were turned upside down, it would be rendered inoperable for its intended purpose." Thus, a modification that renders the prior art device or method inoperable for its intended purpose will not support a conclusion of obviousness. This reasoning is directly on point with respect to the instant claims, because the intended purpose of the Wiesner et al. reference requires knowledge of the amount of sample loaded onto the electrophoresis gel, yet the proposed combination with capillary electrophoresis does not permit such knowledge – any combination involving Wiesner et al. and capillary electrophoresis necessarily requires modification of the quantitative aspect of the Wiesner et al. teachings that will render it inoperable for its intended purpose.

The Examiner's attention is also respectfully directed to *In re Ratti*, 270 F. 2d. 810 (CCPA 1959) (also cited in the MPEP 2143.02 (VI)), in which claims to an oil seal were initially held obvious by the Examiner and the Board of Patent Appeals and Interferences over references including Chinnery et al. In overturning the obviousness decision of the Board, the Court of Customs and Patent Appeals stated:

> "This suggested combination of references would require a substantial reconstruction and redesign of the elements shown in Chinnery et al. as well as a change in the basic principles under which the Chinnery et al. construction was designed to operate.
>
> "Once appellant had taught how this could be done, the redesign may, by hindsight, seem to be obvious to one having ordinary skills in the shaft sealing art. However, when viewed as of the time appellant's invention was made, and without the benefit of appellant's disclosure, we find nothing in the art of record which suggests appellant's novel oil seal as defined in claims 1, 4 and 7." *In re Ratti*, 270 F. 2d. at 813.

Thus, a combination which requires substantial reconstruction or redesign, or which changes the basic principle under which the cited art was designed to operate will not support a conclusion of obviousness. This is on point in the present application, in which combination of Wiesner et al.

with a reference teaching capillary electrophoresis necessarily requires a change in the basic operating principle of the Wiesner et al. reference. In view of this, the proposed combination cannot render obvious the invention as claimed in the subject claims.

Reconsideration and withdrawal of the §103 rejection over this combination of references is respectfully requested.

Myakishev et al. in view of Nolan et al.

Claims 11, 13-17, 28-33, 44-52, 54, 58-64, 66 and 70-73 are rejected as obvious under 35 U.S.C. §103(a) over the teachings of Myakishev et al. in view of Nolan et al. (U.S. 6,287,766). The Office Action states that "Myakishev teaches the method of claims 1-2, 5, 10, 12, 18-19, 22, 27, 34-35, 38 and 43 as discussed above." Myakishev et al. is similarly cited as teaching limitations of independent claims 50 and 62. The Office Action states that "Nolan teaches a method of identifying polymorphisms using flow cytometry." The Office action continues:

> "Regarding claims 13-17, 29-33, 45-50, and 62, Nolan teaches an embodiment wherein unincorporated primers from an initial amplification reaction may be degraded using the heat labile Exonuclease I followed by polymerase extension, where the initial denaturation step destroys the activity of the exonuclease (see for example, column 5, line 60 – column 6, line 20). Nolan also teaches multiplex detection of mutations using multiple sets of primers (column 7, lines 60-63).
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> "Regarding claims 52 and 64, Nolan teaches separation of the amplified products by flow cytometry (column 7, lines 60-63).
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> "Regarding claims 11, 28, 44, 61 and 73, the method taught by Nolan in Example 5 (column 7, lines 1-63) comprises an oligonucleotide ligation assay (OLA) followed by PCR amplification with an upstream primer and a set of downstream primers that may be fluorescently labeled (column 7, lines 11-50). Nolan teaches that the downstream primers each have a different 3' terminal nucleotide (column 7, lines 27-30) in order to identify the polymorphism in a single reaction.
>
> "It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to incorporate the teachings of Nolan into the SNP genotyping method of Myakishev. An ordinary practitioner would have recognized that not all polymorphic sites may be genotyped using only two allele-specific downstream primers, and therefore, would have been motivated by the teachings of Nolan to utilize a subset of four downstream primers each with a different 3' terminus, in order to accurately type these polymorphisms. Also, an ordinary practitioner would have been motivated by the teachings of Nolan to perform a multiplexed analysis,

utilizing multiple primer pairs in order to more rapidly and reproducibly genotype several sites simultaneously. Furthermore, the person of ordinary skill would have been motivated by Nolan to incorporate an exonuclease digestion step following the initial primer extension reaction with the allele-specific primers. Although Myakishev attempted to avoid mispriming by careful design of the allele-specific and energy-transfer primers (see page 164, col. 1), incorporation of the exonucleiase digestion step taught by Nolan following the initial extension would have eliminated the possibility of such mispriming events, and thereby improved the accuracy of the method. Therefore, one of ordinary skill in the art, interested in obtaining a method to more rapidly and accurately determine the identity of multiple polymorphic sites using the method of Myakishev, would have been motivated to use multiple primer sets where the downstream primer sets contain all possible 3' terminal nucleotides and also to incorporate an exonuclease digestion step as suggested by Nolan, thus resulting in the instantly claimed methods."

The rejection is rendered moot with regard to claims 11 and 13-17 and 50-52, 52, 54 and 58-61, by their cancellation herein without prejudice.

Nolan et al. is apparently cited for its teachings regarding the use of a set of downstream primers with differing 3' termini, removal of unincorporated primers, and separation by flow cytometry, none of which remedy the defects of Myakishev et al. with regard to independent parent claims 18, 34 or 62 as amended. Specifically, Myakishev et al. does not teach a method of determining, for a given nucleic acid sample, the identities of the nucleotides at *a set of known polymorphic sites* to be interrogated, in an amplification regimen. With regard to the conclusion in the Office Action that "an ordinary practitioner would have been motivated by the teachings of Nolan to perform a multiplexed analysis, utilizing multiple primer pairs in order to more rapidly and reproducibly genotype several sites simultaneously," Applicant notes, as discussed above and in Dr. Slepnev's declaration, that the method of Myakishev et al. is not readily adaptable to multiplexing. That is, even if Nolan et al. does suggest multiplexing, which is in no way acknowledged herein, the approach does not practically apply to the method of Myakishev et al. As discussed in Dr. Slepnev's declaration, the Myakishev et al. method would not readily adapt to the detection of a set of known polymorphisms in a single reaction because such a multiplex assay would require multiple *distinguishable* fluorescent labels *per polymorphism.* As discussed in the Declaration, in order to be distinguishable, the excitation and emission spectra for each member of each such pair would have to be sufficiently distinct from all other labels so as to avoid interference, quenching or artifactual

signals caused by the interaction of the fluorescent labels. Myakishev et al. teaches only two labels, fluorescein (green) and sulforhodamine (red), which are used to discriminate a single polymorphic site. The reference alone, or in combination with Nolan et al. does not teach an assay to interrogate "the identities of the nucleotides at a set of known polymorphic sites" in one amplification regimen as set out, for example, in independent claims 18, 34 and 62 as proposed to be amended and claims dependent from them. Thus, claims 28-33, 44-49, 63, 64, 66 and 70-73 are not obvious over any combination of Myakishev et al. and Nolan et al.

Applicant also notes that neither Nolan et al. nor Myakishev et al. teaches separating distinctly sized amplification products by size and/or by charge, in an assay that determines the identities of the nucleotides at a set or group of known polymorphic sites in an amplification regimen as required by each of independent claims 18, 34 and 62 as amended.

With regard to the claims subject to the instant rejection and as proposed to be amended, Applicants note that the teachings of Wiesner et al. regarding aliquot removal and agarose gel electrophoretic separation of amplified products for quantitative analysis cannot properly be applied to render the amended claims obvious, for the reasons discussed above and in Dr. Slepnev's Declaration.

Reconsideration and withdrawal of this rejection under §103 is respectfully requested.

Myakishev et al. in view of Nolan et al. and Piggee et al., and Myakishev et al. in view of Nolan et al. and Wiesner et al.

Claims 53, 57, 65 and 69 are rejected as obvious under 35 U.S.C. §103(a) over Myakishev et al. in view of Nolan et al. and Piggee et al. The Office Action states:

> The combined teachings of Myakishev and Nolan result in the instant claims 50, 62 and 64 as discussed above. …..
> Neither Myakishev nor Nolan teaches the use of capillary electrophoresis.
> Regarding claims 53 and 65, Piggee teaches a method of detecting point mutations using capillary electrophoresis and laser-induced fluorescence detection (see abstract).
> Regarding claims 57 and 69, Piggee teaches a capillary electrophoresis device with a sampling device, and a fluorescence detection system (pages 368-369).

> Piggee teaches that the method is fast, avoids radioactive labels and has a high automation potential. …..
>
> It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to utilize capillary electrophoresis as an additional or alternative method of detection in the mutation detection method resulting from the combined teachings of Myakishev and Nolan. Piggee expressly taught the advantages of capillary electrophoresis with a high potential for automation and multiplexing (see above). An ordinary practitioner would have been motivated by the teachings of Piggee to additionally (or alternatively) analyze the SNP detection results using capillary electrophoresis in order to more rapidly and automatically screen a larger number of samples, thus resulting in the instantly claimed methods.

Applicant respectfully disagrees.

First, claims 53 and 57 are cancelled herein, rendering the rejection moot with regard to those claim.

Dependent claims 65 and 69 depend from claim 62, which recites "a method of determining the identities of single nucleotides present at a group of known polymorphic sites," using "an amplification regimen." As discussed above, Myakishev et al. does not teach multiplex analysis – while the authors may report the analysis of nine different SNP targets, at no point is more than one of them interrogated in a single reaction. As also discussed above and in Dr. Slepnev's Declaration, the method of Myakishev et al. is not necessarily adaptable to multiplex analysis. That is, even if Nolan et al. does suggest multiplexing, which is in no way acknowledged herein, multiplexing does not practically apply to the method of Myakishev et al.

Claims 55, 56, 67 and 68 are rejected over a combination of Myakishev et al., Nolan et al. and Wiesner et al. The Office Action acknowledges that "neither Myakishev nor Nolan teaches analyzing an aliquot of the PCR during the reaction process." The Office Action states that "Wiesner teaches a method of determining the initial concentration of a PCR template by measuring the amount of product accumulating in consecutive cycles." The Office Action states that "Wiesner teaches that by analyzing aliquots the need for standardization of the signal due to differing initial product concentrations is omitted" and that "The teachings of Wiesner also permit optimization of cycling parameters and an early identification of the reaction product." The Office Action concludes that it would have been obvious to one of skill in the art to "incorporate the teachings of

Wiesner into the genotyping method resulting from the combined teachings of Myakishev and Nolan." Applicant respectfully disagrees.

First, claims 55 and 56 are cancelled herein without prejudice, rendering the rejection moot as to those claims.

With regard to claims 67 and 68, Applicant again notes that independent parent claim 62 recites a "method of determining the identities of single nucleotides present at a group of known polymorphic sites." However, as discussed above and in the Declaration of Dr. Slepnev, Myakishev et al. does not teach multiplex analysis, and no combination with Nolan et al. remedies that defect. Wiesner et al. also fails to remedy that defect. Thus, neither of claims 67 or 68, which depend from claim 62, can be obvious over any combination of Myakishev et al., Nolan et al. and Wiesner et al.

Finally, Applicant submits that each of independent claims 34 and 62 recites the use of *upstream* **and** *downstream* amplification primers *comprising tag sequences*. This distinguishes each of the references cited in the outstanding Office Action, including Myakishev et al., Piggee et al., Nolan et al., and Wiesner et al. Specifically, claim 34 recites:

> "wherein said amplification regimen is performed using *an upstream amplification primer* ***comprising said first tag sequence***, and a set of distinguishably labeled *downstream amplification primers, each member of said set of downstream amplification primers* ***comprising a said second tag sequence*** comprised by a member of said population of primer extension products and a distinguishable label that specifically corresponds to the presence of a specific nucleotide at said polymorphic site."

Claim 62 recites:

> "wherein said amplification regimen is performed using *an upstream amplification primer comprising* ***the common sequence tag*** *comprised by said first oligonucleotide primer,* and a set of downstream amplification primers, *each member of said set of downstream amplification primers* ***comprising a tag comprised by a member of said set of second oligonucleotide primers*** and a distinguishable label."

Thus, each of claims 34 and 62 requires the use of primers comprising upstream ***and*** downstream tag sequences for the recited amplification. Applicant notes that Myakishev et al. nowhere teaches amplification in which *both* upstream and downstream primers comprise or are complementary to tag sequences. The assertion in the Office Action that the reverse primer in Myakishev et al. *is* a tag sequence (e.g., at page 6 of the Office Action) is not in agreement with the use of the term "tag

sequence" in the specification or the claims. The lack of a teaching of amplification primers comprising upstream ***and*** downstream tag sequences is not remedied by any of Piggee et al., Nolan et al. or Wiesner et al. Thus, no combination of Myakishev et al., Piggee et al. and Nolan et al., with or without Wiesner et al., can render obvious claims 34 or 62 or any claim dependent from them. In view of this, Applicant respectfully requests reconsideration and withdrawal of the §103 rejections of these claims and their dependents over any combination of these references.

In view of the above, Applicant submits that all issues raised in the Office Action are addressed herein. Reconsideration of the claims is respectfully requested.

Should any fee deficiencies be associated with this submission, the Commissioner is authorized to debit such deficiencies to the Nixon Peabody Deposit Account No. 50-0850. Any overpayments should be credited to said Deposit Account.

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Respectfully submitted,

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